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THE SCANNING ELECTRON MICROSCOPY/REPLICA TECHNIQUE AND RECENT APPLICATIONS TO THE STUDY OF FOSSIL BONE

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Abstract

The SEM/replica technique employs high resolution replica materials in order to reflect microstructural details of specimens, such as fossil bones, which cannot be observed directly. The described technique is simple, provides excellent resolution, is maximally adaptable to field and laboratory settings, and is applicable to large and topographically complex bone surfaces. The advent of the technique has made it largely possible to address certain issues in anthropology and paleontology. These contributions have principally been concerned with taphonomy as the study of the bone damage process, and bone biology as it relates to bone growth remodeling processes characterizing the facial growth of our early fossil hominid ancestors.

KEY WORDS: SEM, replication technique, anthropology, paleontology, fossil bone, hominid.

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Introduction

The SEM/replica technique was established 15 years ago by Grundy (1971). It was subsequently reviewed (Pfefferkorn and Boyde, 1974; Pameijer, 1978) and elaborated (Barnes, 1979), thus providing a broader international recognition for the method. This technique involves the production of high resolution replicas employing a low viscosity silicone-based dental impression material to make the negative impression, and an epoxy resin to reestablish the positive structure. The two materials used together comprise a replica combination. The technique makes it unnecessary to subject original specimens to SEM preparation procedures and the electron beam. It also overcomes the size limitations imposed by the SEM chamber when the specimen is large and an insufficient vacuum in the coating unit and SEM chamber is avoided should the original specimen be exceptionally porous or wet. Finally, although it is possible to remove conductive metal coatings on original specimens with sodium cyanide (Sela & Boyde, 1977), the SEM/replica technique circumvents this should it be a problem.

As a direct result of the advantages of the SEM/replica technique, SEM investigations of modern and fossil bone have become routine. In recent years the technique has been reported in several papers describing its utility in anthropology and paleontology (e.g. Shipman, 1981b; Scott, 1982; Rose, 1983; Bromage, 1985b), with special notes on its application in field settings. The technique's contribution to these sciences has principally been in taphonomy (as the study of the bone damage process) and bone growth (as it relates to bone growth processes in fossil hominid craniofacial material), both of which are summarized here.

Replication materials

A most suitable replica combination for fossil bone that I have tested employed an 'Exaflex Injection Type, Vinyl Silicone Rubber Impression Material' (G-C International Corp., U.S.A. Branch, 7830 E. Redfield Rd., Suite 12, Scottsdale, Arizona, U.S.A., 85260) and 'RS Quick-Set Epoxy Adhesive' (RS Components Ltd., P.O. Box 99, Corby, Northants, England, NN16 9RS) (Bromage, 1985a).

These materials were demonstrated to be very adaptable to laboratory and field settings and to provide high resolution replicas efficiently, easily and at relatively low cost. Tests of these materials according to the methodology described below demonstrated their effectiveness in replicating details on the order of 0.1-0.3 microns on both large and three-dimensionally complex fossil bone surfaces. Since the time of this published replica combination (ie. Bromage, 1985a), however, the epoxy formula has changed and subsequent material tests have demonstrated an absolute replicating limit of 0.3 microns.

McCabe & Storer (1980), speaking from the clinical setting, say that "the choice of material for a particular clinical situation can be made by considering the physical property requirements demanded by that situation" (p. 77: emphasis mine). This is likewise important when working with rare or even unique fossils. If the fossil specimen is three-dimensionally complex and/or it is very fragile, then a silicone impression material with a very low tensile strength is required. If the original is very porous, a higher viscosity is required in order that the material will not penetrate into the specimen, risking exfoliation of the surface when the replica is pulled away. However, it is not always practical to have a replica material for every possible situation and so, for best results, a low viscosity material with low tensile strength is needed (e.g. Exaflex), and should relatively high viscosity be required, the material can be mixed and permitted to begin to set ever so slightly and then applied to the original. There is a consequent loss of some of the finest microscopic details but this is the compromise that must be made with important and fragile specimens. In some cases viscosity can be increased by mixing in a greater proportion of the more viscous hardener paste but this will, of course, give rise to a shorter cure time.

Rapid cure (e.g. 5 min.) epoxies are quite useful for making positive replicas. Their relatively high viscosity ratings make retaining walls, which are required for 24 hour cure epoxies, unnecessary. Objects can therefore be replicated even in their complex three-dimensions and very thin replicas can be produced (e.g. 1 mm or less), thus limiting the amount of epoxy required. The rapid cure epoxy resins of the kind commonly purchased at a hardware store (for repairing china, metals, glass, etc.) are most suitable. Their ease of handling, their potential ability to provide excellent surface detail, their relatively negligible toxicity and efferent vapors and their relatively low cost make them attractive in a replica combination.

Whereas the Exaflex and RS epoxy materials make a capable replica combination, these materials may not be preferred nor available everywhere. Materials testing is thus a necessary exercise for anyone wishing to employ the SEM/replica technique. Furthermore, it is irrelevant, for instance, that RS epoxy may not provide excellent surface detail in tests with other impression materials. Indeed, this has often been my experience and it reinforces the claim that materials testing is vital to the performance of the technique. Researchers should be encouraged to perform materials tests with their own research problems in mind.

Replication methods

The methods of mixing and applying silicone-based impression materials and epoxy resins are largely acquired skills. The following points bear special relevance to fossil specimens.

Once mixed, the impression material can be applied to the specimen by small pressing movements of the spatula tip up and down while slowly maneuvering the material linearly and allowing more material to be added from the tip, or by new additions of mixed material. The pressing movements, much like little "push-ups", are important for pushing the impression material into very small spaces, and the extension linearly, with the width dimension of the spatula tip at some angle to the surface (never parallel), is important for displacing air potentially trapped in small depressions.

Environmental conditions are known to affect the cure time and so it is wise to keep checking remnants of material on the mixing pad before attempting to remove the replica. If the specimen is large, cummulative additions of material can be made to the cured replica remaining in place. It is wise to keep each subsequent application within the limits of the situation - governed by topography, quality of the specimen surface, experience, etc. This will usually reach a maximum of 3-5 square centimeters per application. Specimens can normally be replicated in their entirety and in one piece (Fig. 1). This avoids the problem of overlapping sub-unit replicas which can make precise mapping of surface details difficult later. After the material has cured, it is removed by hand or by wood or plastic tools, never by metal utensils that may damage the fossil surface. The elastic property of the material can also be used to deform the replica gradually so that it displaces itself from the surface.

The positive replica is produced by placing a small amount of the mixed epoxy on the negative impression and then spreading and pressing it in with compressed air. This "air" is frequently used in the photographic industry to dust off negatives, etc., and is composed of chlorodifluoromethane. An air compressor fitted with an in-line oil filter can also be used. The layer is pressed very thin (e.g. 10-100 microns) and then additional epoxy from the same as yet uncured mixture or new mixtures can be added for support. This technique permits the epoxy, which has a relatively high viscosity rating, to enter into the smallest pores and spaces and assists in the replication of microscopic details.

When the positive replica has cured, it is still anelastic for a time and can be conveniently cut into pieces with sharp scissors or a razor blade, suitable for observation with the SEM. The lines of sectioning can be drawn onto another 3-D epoxy positive (most easily with a high-density fine-tipped water soluble pen) as a reference while observing the separate pieces with the SEM, if this is convenient. According to this methodology, large three-dimensional and topographically complex surfaces such as facial skeletons (Fig. 2) and mandibles can be replicated.

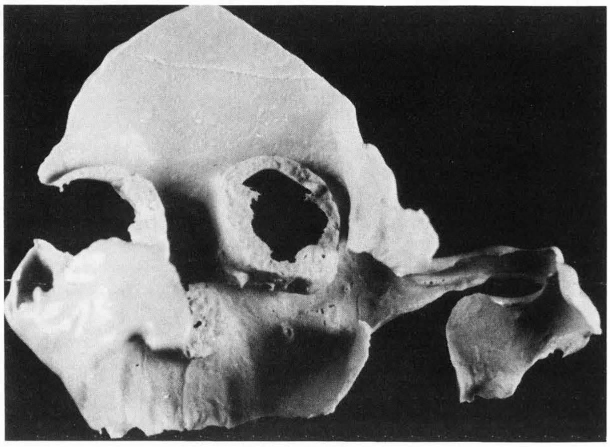

Figure 1. Whole face silicone impression of the Taung child, an early hominid of the genus Australopithecus.

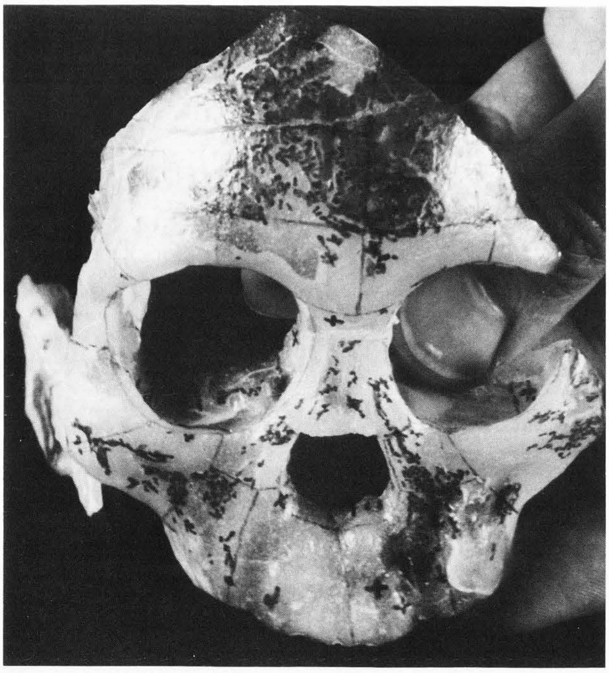

Figure 2. A high resolution whole face epoxy positive of the Taung child. Irregular ornament represents precise mapping of remodeling activities and lines demarcate the sectioned pieces for the SEM copy of the Taung face.

Usefulness of the SEM/replica technique in studies of fossil bone

Two principal lines of investigation utilizing the SEM/replica technique have been pursued in fossil bone studies: taphonomy and bone biology. Taphonomy, "derived from Greek terms meaning 'the science of the laws of embedding'" (Behrensmeyer, 1984:558)), is a broad field of which one of the specialties, microscopic taphonomy, is rapidly gaining in the literature, largely as a result of the advantages of the SEM/replica technique.

Fossil bones lead a very tenuous existence indeed. Their preservation potential, or rather their probability of surviving to discovery, depends entirely on the nature of the predepositional, depositional (or transport) and diagenetic (in situ) phases in its "life" history. These phases have unique properties which are more or less specific to the local environmental (atmospheric, geological and biological) agencies. Particular emphasis has therefore been placed on the role of microscopic taphonomy in 1) paleoenvironmental reconstruction (Bromage, 1984a) and 2) on the identification of hominid "signatures" in the form of cutmarks on bone (Shipman, 1981a,b).

Experimental studies have confirmed that the impact energy of small particles in moving water can lead to the rounding of bone edges (Shipman, 1981a). The shiny surfaces of bones abraded by air-borne particles have been shown to be due to the many perfectly smooth facets impressed into the bone from their impacts (Bromage, 1984a), rendering a discontinuous surface. In this same study it was concluded that rough surfaces characterized weathering processes (atmospheric) and smooth surfaces characterized postdepositional taphonomic processes. These processes were implicated in bone surface changes on replicas of fossil hominid craniofacial bone.

Cutmarks on fossil bone represent hominid activities concerned with the procurement of animal tissues and as such is a 'signature' of the hominid behavioral complex. Initially it was thought that cutmarks left by the intentioned manipulation of a bone or stone tool were characteristic at the microscopic level from other such marks (Potts & Shipman, 1981). Cutmarks have subsequently been shown to be significantly different from carnivore and rodent gnawing, thus making it possible to identify biological agencies responsible for various marks on bone, at the microscopic level (Shipman & Rose, 1983).

Bromage and Boyde (1984) were able to show that the directionality of cutmarks could be determined and that not all marks made by a single tool under similar conditions were the same. It was also pointed out in this paper that different bone tissue types, such as that described for bovids and carnivores (Enlow & Brown, 1958), react differently to taphonomic agencies.

Recently it has been shown that marks caused by trampling can mimic intentioned cutmarks (Andrews & Cook, 1985; Behrensmeyer et al., 1986), indicating that more reliance would have to rest upon patterns of mark distribution, bone breakage patterns and site formation (in the archeological sense). However, there now exists a feature of intentioned cutmarks that is distinguishable from trampling marks and which is related to the directionality criteria set out by Bromage and Boyde (1984). This feature is handedness. It is normally assumed that cutmarks and trampling marks are similar because of the similarity in the physical dynamics of the process (Behrensmeyer et al., 1986), but I believe that the precision of the human hand is not normally duplicated in nature by other cutting processes.

Bone in its fresh or its superficially anorganic state behaves like a brittle solid in response to the cutting process (Bromage & Boyde, 1984). This process is accompanied by stress fracturing and chipping that reflect directionality and handedness information during a controlled cutting movement. Figure 3a and 3b illustrate left and right handed cutmarks, respectively, made on glass with a flint tool. Directionality is indicated by Hertzian fracture cones; the bases of the cones face the cutmark direction. Handedness is indicated by the more abraded lateral (to the hand) wall as opposed to the more regular medial wall. Polarised light investigation of these cutmarks revealed fracture cones on both sides of the marks but the outer face of the tool, inclined over the substrate due to a supinated hand, came into contact with the lateral wall of the cutmark and abraded these fracture cones away.

Figure 4 is a replica of a cutmark produced on fresh calf long bone with the periosteum intact. Directionality and handedness are illustrated as described above (left image) with the additional illustration (right image) of a directionality indicator called 'bone smears' (Bromage & Boyde, 1984) lifted and facing rearward of the cutmark directionality. Although I have witnessed directionality on many trampling marks, these marks do not normally contain handedness information and certainly do not demonstrate handedness consistently along most of the length of the marks. Apart from the obvious advantage to the study of handedness in early man, I would propose that handedness of cutmarks may be used as a discriminator of cutmarks when the etiology of these marks is in question.

Taphonomy for the sake of reconstructing past bone biological processes is another most useful line of investigation. For such a study it is not so important to know what taphonomical processes damaged the bone surface, but simply to understand that the surface has been damaged. This understanding prevents an assessment of bone damage as a true biological surface or phenomenon. Bromage (1984a) undertook such a study on bone surfaces undergoing normal bone growth remodeling at the time of death. These surfaces are broadly characterized by the presence of incompletely mineralized collagen fiber bundles (forming) or resorption lacunae (resorbing) (Boyde & Jones, 1972).

By experimentally abrading these surfaces it was possible to begin to "understand" that 1) the surface was indeed altered (damaged), 2) that this damage was distinct from the appearance of normal bone surfaces and that 3) once the damage was understood in this way it was possible to "read through" the damage, as if it were "noise" in the system, to see the biological information underneath. It was discovered that incompletely mineralized collagen fiber bundles characteristic of forming surfaces were abraded most readily but that there was a larger and more resistant feature of these surfaces, called intervascular ridging (IVR) bone (Fig. 5) which survived moderately abrasive conditions.

Thus, as a result of these studies, it was possible by means of the SEM/replica technique to investigate bone growth remodeling on fossil hominid craniofacial remains (Bromage, 1984b, 1985b, 1986, 1987). The methodological variations and results of this study are summarized here. Prior to SEM analysis, the metalized hominid replicas were observed with a light binocular microscope at magnifications of 10 to 40 times, at which time IVR bone could be identified. Thus it was possible to make a preliminary map of the extent of forming bone surfaces. These IVR bone surfaces were recorded on the three dimensional model of the specimen prepared for this purpose using a high-density fine-tipped water soluble pen. These surfaces were then verified during SEM analyses of the specimen (Fig. 6).

It should be noted, however, that considerable experience with the appearance of abraded forming bone surfaces was essential to these analyses. Often, forming bone surfaces were identified on the basis of visible tracts of osteocyte lacunae and collagen fiber bundles. These surfaces were usually interpreted with the SEM as fully mineralized collagen fiber bundles without mineralized superficial interfiber bundle matrix, and half-formed osteocyte lacunae. This surface on a fresh bone sample would ordinarily be interpreted as a resting bone surface, or possibly mineralizing to the level of resting osteoblasts, but as resting bone cannot be interpreted separately from surfaces characterized by active formation which have been abraded, the interpretation of all forming bone surfaces on fossil bone specimens must simply be that the last bone growth activity state was a forming one.

Subsequent SEM analysis permitted verification of IVR bone previously interpreted with the light microscope. Also at this time Howship's lacunae characteristic of resorptive surfaces were interpreted and mapped onto the model using a different colored pen. This characteristic

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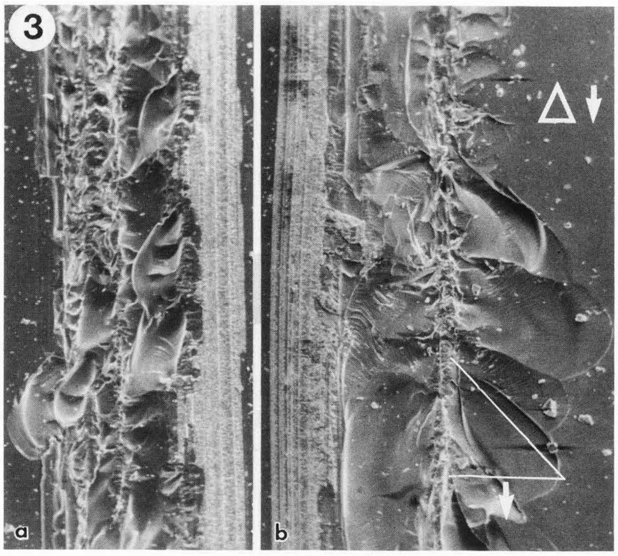
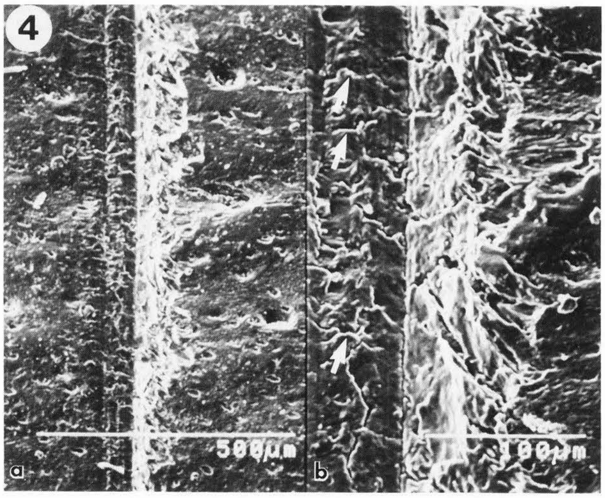
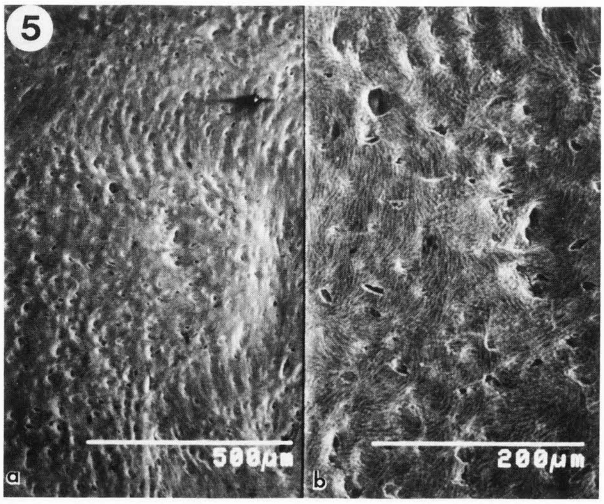
Figure 3. Left (a) and right (b) hand cutmarks on glass. Directionality is indicated by Hertzian fracture cones (bases of the cones facing the cutmark direction) and handedness is indicated by rough lateral margins. Field widths 0.9 mm (a) and 1.3 mm (b).

Figure 4. Left image (a) is a replica of a cutmark on bone: directionality is from top to bottom and the cutmark was made by a right handed person. Right image (b) is a higher magnification view of the cutmark illustrating bone smears on the cutmark floor (arrows).

Figure 5. Replica of intervascular ridging bone (IVR) on superficially anorganic immature macaque maxilla: low (a) and high (b) magnification views.







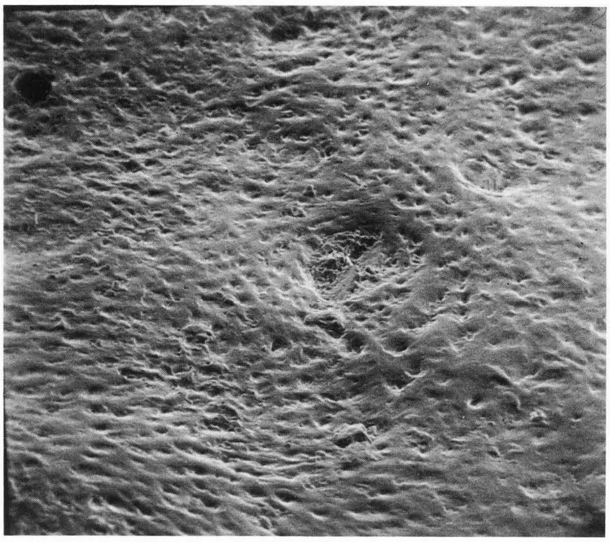


Figure 6. Replica of IVR bone from the mandible (Sts 24) of a member of the genus Australopithecus. Field width 0.75 mm.

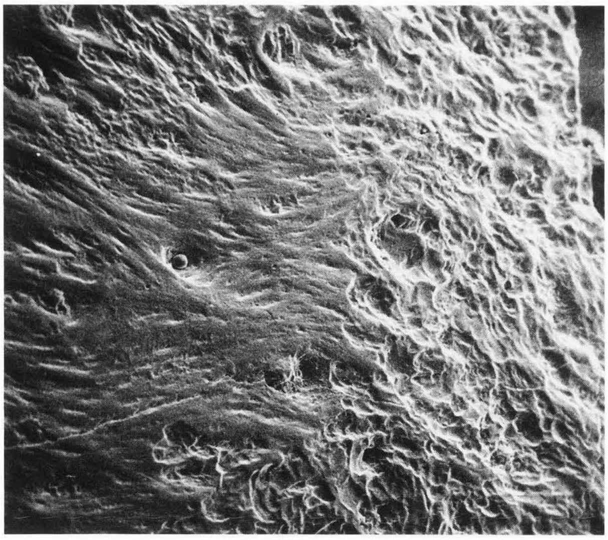


Figure 7. Replica of resorption lacunae (right) and IVR bone (left) from the mandible (SK 64) of a member of the genus Paranthropus. Field width 1.35 mm.

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pitting was readily identifiable on replicas of fossil hominid bone (Fig. 7). Resorption lacunae were only assessed with the SEM because of the difficulty of interpreting resorbed from abraded bone surfaces by means of light binocular microscopy. Furthermore, as was the case for interpretations of forming bone surfaces, no attempt can be made to identify resting resorbed surfaces and the interpretation must simply state that the last bone growth activity state was a resorptive one.

Recently I compared early hominid facial remodeling patterns and interpretations of facial growth to those of Pan and modern Homo (Bromage, 1986). It was determined that Australopithecus (represented by 10 immature facial remains) and early Homo (represented by 4 immature facial remains) shared the primitive facial remodeling pattern as represented by the extant chimpanzee but differed in their rates of remodeling activities, accounting for the variable extent to which members of these taxa exhibited a prognathic and ape-like facial profile. Nevertheless, australopithecine and early Homo facial growth was characterized by bone deposits on all anteriorly-facing aspects of the face which served to emphasize the anteriorward mode of growth. This was combined with an anteriorly-drifting pterygoid complex, thus allowing the full complement of deposits at the maxillary tuberosity to be translated into anterior displacement of the midface.

Paranthropus (represented by 16 immature facial remains) exhibited a facial remodeling pattern in parallel with modern Homo, accounting for the relative orthognathy of this taxon. Paranthropines were characterized by resorption over the nasoalveolar clivus and the deciduous canine-molar region of the mandible during ontogeny. These remodeling features were combined with marked increases in posterior facial height, an inferiorly-drifting pterygoid complex, a relatively deep posterior palate and an upward rotation of the upper face compensated for by an anterior relocation of the upper face above the jaws. An inferiorly-directed facial growth vector was the result and, combined with posterior relocation of the jaws, determined an ontogenetic sequence related to the development of an anterior and vertically disposed masticatory system.

It has thus been realized that early hominid craniofacial morphology can be expressed as a function of bone growth remodeling processes. Just as facial remodeling differences between modern Homo and Macaca reflect differences in their respective craniofacial morphologies (Enlow, 1966; Duterloo & Enlow, 1970), so do they reflect differences between early hominid taxa. This demonstrates the taxonomic valency of facial remodeling whether it be due to the demonstrated differences in the patterns of remodeling activities, as between Australopithecus and Paranthropus, or due to the differences in the rates of remodeling activities, as between Australopithecus, Pan and early Homo (Bromage, 1986).

Conclusions

The SEM/replica technique is a means of investigating the intact surfaces of specimens with an SEM when the researcher, for one reason or another, cannot or does not want to subject the original specimen to SEM preparation procedures and the electron beam. Firstly, the researcher must know and understand the physical and chemical properties of his/her specimens. Secondly, this will determine the physical and chemical nature of the replica combination to be used on such a specimen. Thirdly, this requires an exercise in materials testing in order to find the right materials for the right job.

There can be no doubt that the SEM/replica technique has enabled researchers of fossil bone to pursue specialized studies in microscopic taphonomy and developmental anatomy that would otherwise be limited. The progress in these areas has been fruitful and is having a significant impact on interpretations of paleoenvironments, early hominid behavior and bone biological processes millions of years ago.

Future directions in microscopic taphonomy must surely be to identify the nature of specific taphonomic agencies. Most researchers in this field presently ignore the fact that bone is a unique biological-structural material that responds uniquely to taphonomic processes. Thus the current precedent treats bone as an isotropic or neutral substance in taphonomic studies. An appreciation of these facts could result in the recognition of much important data and a more complete understanding of the processes acting on bone.

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Editor's Note: All of the reviewers' concerns were appropriately addressed by text changes, hence there is no Discussion with Reviewers.